Mitochondria are the energy producing organelles of eukaryotic cells. Owing to their endosymbiotic evolutionary history, they contain their own genome (mtDNA) that encodes for thirteen proteins essential for ATP production.
In mammalian cells, multiple mtDNAs are compacted into protein-DNA complexes called "nucleoids". A major component of these nucleoids is the mitochondrial transcription factor A (TFAM), a member of the high mobility group (HMG) family of proteins. This abundant protein binds DNA with little sequence specificity, and is able to coat the entire mtDNA molecule. It not only serves a role in mtDNA packaging, but is also required for mitochondrial transcription. At this point, dynamics of the TFAM-DNA interaction remain unclear.
Experiments on single DNA molecules offer a very direct way to study TFAM dynamics. Tethered Particle Motion (TPM) experiments show that the system quickly equilibrates with the buffer, and that the end-to-end distance of the DNA decreases upon TFAM binding. Manipulations of single DNA molecules with two optical traps offer an explanation: TFAM decreases the DNA's stiffness (persistence length). A possible molecular mechanism for this decrease is that TFAM introduces bends in the DNA.
Adding single-molecule fluorescence to the dual optical trap illuminates TFAM's binding behavior. Literally seeing TFAM on the DNA, we can derive on- and off-rates, and determine that TFAM does not bind cooperatively. Interestingly and seemingly unrelated to its role in DNA organization, we also observe that TFAM can rapidly bind single-stranded DNA (ssDNA), but not when the ssDNA is under tension. The physiological function of TFAM binding to ssDNA could be related to its regulation of transcription.

## Symposium 9: Biophysics of the Failing Heart

**1153-Symp**
**What is the Effect of A Familial Hypertrophic Cardiomyopathy Mutation on Cardiac Myosin Function?**
**Susan Lowey**.
University of Vermont, Burlington, VT, USA.
Familial hypertrophic cardiomyopathy (FHC) is a clinically and genetically heterogeneous disease which is a major cause of heart failure. The landmark discovery that a point mutation at residue 403 (R403Q) in the β-myosin heavy chain (MHC) can cause a lethal form of FHC was made in 1990, but the effect of this mutation on the functional properties of human cardiac myosin remains poorly understood. One problem has been that the prevalent mouse model for FHC expresses predominantly α-MHC. The β-MHC, however, is the predominant isoform in the ventricles of all larger mammals. Even though the α- and β-MHC share $>$ 90 % sequence identity, they differ ~ 2-fold in enzymatic and mechanical properties, raising the possibility that the effect of a disease mutation may depend on the isoform backbone. To address this question we used a transgenic mouse model in which the endogenous α-MHC was replaced with transgenically encoded β-MHC. A His-tag was cloned at the N-terminus of α- and β-MHC, along with the R403Q mutation, to facilitate isolation of myosin or its head subfragment-1 (S1).We find that the steady-state ATPase activity and *in vitro* motility of mouse α-MHC is enhanced by the R403Q mutation, as reported previously, but the R403Q mutation in a β-MHC background shows a slight reduction in activity. A more in-depth analysis of the R403Q phenotype is being undertaken by stopped-flow kinetics to measure the nucleotide turnover in these mutant S1 isoforms. In order to determine the extent of species-dependent differences, we are comparing the functional properties of β-cardiac myosin in the mouse with those in the rabbit, a model system which more closely resembles humans in protein composition and disease phenotypes.

**1154-Symp**
**Reduced Responsiveness to β-Adrenergic Agonists in Murine cMyBP-C Cardiomyopathy**
**Richard L. Moss**, Carl Tong.
Univ Wisconsin Sch Med, Madison, WI, USA.
Myosin binding protein C (MyBP-C) is a thick filament accessory protein that has both structural and regulatory roles in striated muscle contraction. We are studying the roles of the cardiac isoform of MyBP-C in mouse models in which the cMyBP-C gene has been disrupted, resulting in ablation of the protein, and in mice expressing mutant protein in which residues that are phosphorylated *in vivo* by PKA have been replaced with ala or asp. Ablation of cMyBP-C results in a cardiac phenotype similar to many inherited cardiomyopathies in humans, i.e., septal hypertrophy, increased arrhythmic activity, and systolic and diastolic dysfunction. Studies of isolated myocytes from wild-type and null mice suggest that cMyBP-C regulates the kinetics of cross-bridge interaction with actin, a mechanism that is lost in the null mouse. Studies of myocytes from mouse lines expressing phosphorylation mutants of cMyBP-C indicate that PKA stimulation of contraction kinetics in myocardium is in large part due to phosphorylation of cMyBP-C, which appears to relieve a structural constraint on myosin and increases the likelihood of myosin binding to actin. Living myocardium expressing non-phosphorylatable cMyBP-C was found to exhibit depressed twitch force-frequency relationships and reductions in both frequency-dependent and β-agonist- induced acceleration of relaxation. These results can be explained by a model in which cMyBP-C phosphorylation accelerates cross-bridge interaction kinetics in wild-type myocardium, a regulatory mechanism that is lost in myocardium expressing the non-phosphorylatable mutant cMyBP-C. Supported by NIH R01 HL082900 and P01 HL094291.

**1155-Symp**
**The Giant Elastic Protein Titin Role in Muscle Function and Disease**
**Henk Granzier**.
University of Arizona, Tucson, AZ, USA.
No Abstract.

**1156-Symp**
**Rescue of Familial Cardiomyopathies by Modifications at the Sarcomere Level and $Ca^{2+}$ Fluxes**
**Beata M. Wolska**.
University of Illinois, Chicago, Chicago, IL, USA.
Familial cardiomyopathies are commonly linked to missense mutations, deletions or truncations in sarcomeric, cytoskeletal, or intermediate filament proteins and give rise to hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) or restrictive cardiomyopathy. Although in the last two decades much information about the pathophysiology of genetically linked HCM and DCM has been provided by studies using transgenic animal models, there is still no therapy to prevent the development of the disease and increase survival in patients with HCM or DCM. Our emphasis here is on development of new therapies for treatment of HCM and DCM linked to mutations in thin filament proteins that are associated with increased and decreased myofilament sensitivity to $Ca^{2+}$ respectively. We hypothesize that direct modifications of myofilament $Ca^{2+}$ sensitivity and/or alteration in $Ca^{2+}$ fluxes can serve as new therapeutic targets. Therefore if 1) HCM is associated with increased myofilament sensitivity to $Ca^{2+}$, interventions that desensitize the myofilament to $Ca^{2+}$ may serve as potential new therapeutic targets and 2) DCM is associated with decreased myofilament sensitivity to $Ca^{2+}$, interventions that sensitize the myofilament to $Ca^{2+}$ may serve as potential new therapeutic targets. There are several possible targets within myofilament proteins for altering myofilament $Ca^{2+}$ sensitivity, in particular troponin I. Alterations in $Ca^{2+}$ regulation by modification of sarcoplasmic reticulum Ca-ATPase (Serca2) or phospholamban levels are additional potential targets for HCM and DCM.

## Minisymposium 2: Nanomedicine: Biophysical Approaches to Clinical Problems at the Nanoscale

**1157-MiniSymp**
**4.0 Å Cryo-EM Structure of the Mammalian Chaperonin: TRiC/CCT**
**Yao Cong**[1], Matthew Baker[1], Joanita Jakana[1], David Woolford[1], Stefanie Reissmann[2], Steven J. Ludtke[1], Judith Frydman[2], Wah Chiu[1].
[1]Baylor College of Medicine, Houston, TX, USA, [2]Stanford University, Stanford, CA, USA.
TRiC is a eukaryotic chaperonin essential for *de novo* folding of ~10% newly synthesized cytosolic proteins, many of which cannot be folded by other cellular chaperones. Unlike prokaryotic and archael chaperonins, each of its two rings consists of eight unique, but similar subunits. Using single particle cryo-EM, we determined the mammalian TRiC structure without any symmetry imposition at 4.7 Å resolution, which is the highest resolution asymmetric cryo-EM reconstruction to date. An analysis of this map allowed us to elucidate the relative orientation of the two rings and the two-fold symmetry axis location between them. A subsequent two-fold symmetrized map yielded a 4.0 Å structure, in which a large fraction of side chains and structural elements including loops and insertions appear as visible densities. These features permitted unambiguous identification of all eight individual subunits, despite their similarity. A Cα backbone model of the entire TRiC complex was subsequently refined from initial homology models against the cryo-EM density based on our subunit identification. A refined all-atom model for a single subunit